

# HERPESVIRUS HOMINIS (VIRUS OF HERPES SIMPLEX)<sup>1</sup>

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## INTRODUCTION

The use of the word Herpes, as applied to certain manifestations of skin disease, has a long tradition, starting with Hippocrates. The many interpretations of its meaning through 25 centuries were discussed in a scholarly review by Beswick (9), who pointed out that the first author to give a clear account of the disease Herpes Febrilis was Richard Morton in 1694, but that it was not until the end of the 19th century

that the modern concept finally was accepted. After the nomenclature of the disease was established, the causative agent, when isolated in 1912, came to be known as the Virus of Herpes Simplex. In 1953 the taxonomy of viruses became a subject of concern, and the virus received the designation "*Herpesvirus hominis*" (2), a name which has not received general acceptance in the literature. It seems probable that, with the continued discovery of new viruses from other species, which have the characteristics of the herpesvirus of man, and with further study of viral structure, a more useful nomenclature will be devised. [See Melnick et al. (44) and Wildy (84).]

The study of the virus has suffered from the interference of two world wars. World War I

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started just at the time that Grüter had demonstrated that the virus could be isolated from the dendritic ulcer of man, and that it would cause a similar lesion in the rabbit. Communication was virtually shut off until 1919, when Löwenstein repeated Grüter's work and extended it to the isolation of the same virus from the herpes vesicle of the skin. As a supposed cause of Von Economo's encephalitis, epidemic for about 10 years after World War I, the virus was studied extensively during the 1920's but this interest died down in the 1930's, when it became clear that Von Economo's disease was not caused by this virus. At this time, the role of the virus as a disease agent came in question, because it was found to cause its recognized clinical manifestation of "fever blisters" only in those individuals who had circulating antibodies, and therefore did not act like an infectious agent in the usual sense. The problem was clarified in 1939 when it was demonstrated that the virus caused stomatitis, as a primary infection, in those without antibodies, while the recurrent disease of "fever blisters" occurred only in those with circulating antibodies after recovery from the primary infection. This important milestone in virology failed to excite major interest because of the more pressing health problems imposed by World War II. These early studies were considered in extenso by Van Rooyen and Rhodes (79). Since the end of the war, interest in the virus has increased rapidly. The work during the earlier postwar years was reviewed by Stoker (72) and Weisse (82). This review will be directed chiefly to a description of the fundamental aspects of research on the virus. The following subjects will be considered: structure, biochemistry, mechanism of cell infection, cytology, serology, latent infection, and recent observations of clinical interest.

#### STRUCTURE

The introduction of the technique of negative staining with phosphotungstic acid for electron microscopy (10) provided a new understanding of viral structure. Wildy and Watson (85) studied the morphology of the virus by use of this technique. The virus particle ("virion") consists of a roughly spherical central "core" measuring 750 Å in diameter, within which is contained the virus nucleic acid, now firmly established as deoxyribonucleic acid (DNA)

(56, 8). Surrounding the core is the stable capsid, which has the characteristics of an icosahedron with a 5:3:2 axial symmetry. This measures 1,000 Å in diameter and is composed of 162 capsomeres, which are elongated structures, 90 to 100 Å in diameter by 120 to 135 Å in length. To fit the symmetrical arrangement, 150 of them are hexagonal and 12 are pentagonal in cross section. Many virions have envelopes, derived from host-cell membrane, which surround the capsid and bring the size of the particle to 1,450 to 2,000 Å in diameter. As seen under an electron microscope, virions vary in the completeness of their structure; there are particles complete with envelopes ("enveloped particles") and a central core containing nucleic acid ("full" particles); particles with envelopes but an empty central core ("empty" particles); and particles without envelopes ("naked" particles), some with "full" and some with "empty" cores (80). Sections of infected cells, fixed in osmium and embedded in Aquon, show a dense eccentric oval or rod-shaped body, 350 Å in width, lying in the center (core) of the virion. This is known as the nucleoid and is deoxyribonuclease-sensitive (19). The host nature of the envelope was shown chemically by Epstein and Holt (20), who demonstrated the presence of adenosine triphosphatase both in the envelope and in the host-cell membrane, and immunologically by Watson and Wildy (81), who reported the agglutination of enveloped particles by antihost serum and of naked particles by antiviral serum and not vice versa.

#### BIOCHEMISTRY

##### *Composition of the Virus*

Russell et al. (60), in a preliminary report, described the composition of the virus as 100 parts DNA, 25 parts carbohydrate, and 320 parts phospholipid, to 1,000 parts protein. The DNA is double stranded and denser than that of the host cell, 1.727 g/ml as compared with 1.710 g/ml (57). It has a sedimentation constant of 44S and a molecular weight of  $68 \times 10^6$ . The bases of the viral DNA are arranged in different proportions as compared with those of the host DNA; the guanine-cytosine to adenine-thymine ratio is 68% in viral DNA and 42% in host DNA. Each viral particle is estimated to contain  $1.2 \times 10^{-10}$  µg of DNA (58).

### *Changes Induced by Infection*

After infection of cells in suspension, under one-step growth conditions, the following biochemical changes were observed (59). Within the first 2 hr, specific viral complement-fixing (CF) antigens appear and there is an increase in the activities of two enzymes (DNA nucleotidyl transferase and deoxyribonuclease I) associated with DNA synthesis. DNA precursors reach a critical level by the fourth hour after infection, and by the fifth hour "heavy" viral DNA can be detected. The formation of this continues until the seventh hour and then levels off. Viral particles, seen shortly after the appearance of DNA, increase in number following a similar curve. Infectivity, however, does not become detectable until the sixth hour, after which it increases rapidly until the ninth hour and then levels off.

### *Induction of Enzymes*

Dubbs and Kit (17) studied the thymidine kinase system in infected mouse fibroblast cells. This enzyme, important in the phosphorylation of thymidine, was found to be absent in a mutant cell line, LM(TK-). When this cell line was infected with herpesvirus, the synthesis of thymidine kinase was induced. However, two mutants of the infecting strain of herpes were found which lacked this kinase-inducing activity.

### MECHANISM OF CELL INFECTION

Infection of the host cell and multiplication and release of newly formed virus from the host cell can be discussed in sequential steps.

#### *Adsorption*

Both "enveloped" and "naked" particles can be adsorbed to the host cells. The "enveloped" particles appear to be adsorbed more readily than the "naked" particles (29). Smith (67) produced evidence that only the "enveloped" particles actually multiply in the HEp-2 cells he apparently used. However, Wildy and Watson (85), using a baby hamster kidney cell line (BHK 21), calculated that the "naked" particle must also be infectious. These authors also discussed the paradoxical situation wherein the antiviral serum, which neutralizes the infectious "enveloped" particles, cannot agglutinate them.

#### *Penetration*

Epstein et al. (21) described the events that follow attachment of the virus particle to the

host cell. The host cell villi enfold the particle, which is drawn into a pinocytotic vesicle. It then travels in a series of vesicles towards the nucleus, during which time the "enveloped" particle is gradually stripped of its envelope by a proteolytic cell enzyme. [The paradox of destruction by a host-cell enzyme of the envelope derived from the host may be explained by the work of Roane and Roizman (53), who demonstrated that there was a change in antigenic structure of the host-cell membrane during the course of infection. The envelopes therefore do not completely present the original host-cell structure.] In the paranuclear area, the stripped particle is released from the vesicle by some mechanism not yet clear but which seems to be specific, since gold particles, which are ingested in a similar fashion to the virus particle, are not so released. The next step has not been demonstrated but must be the release of viral DNA, which then enters the nucleus where replication of new virus takes place.

### *Multiplication*

The development of new virus has been studied in sections of infected tissue. The earliest particles appear as small, dense spherical bodies, 300 to 400 Å in diameter, known as "primary bodies" (45). These are scattered through the nucleoplasm and sometimes are arranged in a crystalline array. These bodies correspond in size to the nucleoid (see Structure). In the nucleus, these acquire a single membrane which is considered to be the protein capsid.

The central portion of the nucleoplasm appears, from electron micrographs, to be the site of viral multiplication; this has been confirmed by Munk and Sauer (46). These investigators demonstrated that thymidine labeled with tritium given at the same time as the virus was concentrated in the central portion of the nucleus, whereas, if the labeled thymidine was given 18 hr prior to infection, the label was concentrated in the subsequently margined nucleoplasm. In the nucleus, the primary particles enlarge and acquire a membrane and are next seen in smooth-walled vesicles near the nucleus, possibly dilatations of the Golgi apparatus (19). The mechanism of release of particles from the nucleus has not yet been clarified. In the cytoplasm, the particles appear to achieve maturity. It has been estimated that one infected cell produces 1,000 new viral particles, of which 5 to 10% are infectious (58).

### *Release of Virus*

Infected HeLa cells are not disrupted by the virus, but particles leak out of the cell by evaginating the membrane surrounding the vesicle or that of the cell itself. Thus, they acquire a triple-layered envelope which, under an electron microscope, appears identical to that of the host-cell membrane (19). It seems possible that with other cell lines, e.g., BHK 21, virus disrupts rather than leaks out of some of the infected cells so that numbers of "naked" particles are also freed into the medium (85).

### CYTOLOGY

Infection with herpesvirus leads to a number of different changes in the infected cell.

#### *Development of Inclusion Body*

This has been the hallmark of herpes infection since it was first described by Lipshütz in 1921, and has been studied by several authors. The most recent and detailed study was by Love and Wildy (41). They used Toluidine Blue-molybdate stain on HeLa cells infected with a high-multiplicity inoculum, and described the series of events that follow the entrance of the virus into the cell. The first change, which occurs within 30 min after infection, is an enlargement of the nucleoli due to the increase of ribonucleoprotein (RNP) bodies (nucleolini). These are subsequently extruded from the nucleoli into the nucleoplasm to form RNP bodies, called B bodies to distinguish them from the A inclusions which develop later. By 3 hr after infection, RNP from the pars amorpha begins to diffuse into the rarified nucleoplasm that surrounds the nucleoli where, almost immediately, within 30 min, the classical inclusion (A body) begins as small masses of deoxyribonucleoprotein which subsequently fuse. As the inclusion grows, it displaces the nucleoli until they disintegrate or become diffusely dispersed. It can be assumed that the invading viral DNA both serves as a template for new DNA synthesis and induces the formation of messenger ribonucleic acid (RNA) in the nucleoli. This determines the specific structure of the viral protein and the induced enzymes required for its formation. The viral protein is apparently synthesized in the nucleus, since the first membrane (capsid) that surrounds the "primary body" can be seen in the nucleoplasm under an electron microscope. The large DNA-containing inclusion body is not all new

virus, but probably can be accounted for by the excess of DNA that is made in infected cells over and above that required for the manufacture of viral particles (48). The virus particles appear, in the otherwise homogenous A body, as spherical solid or hollow granules (A granules). These granules make their first appearance at the time the infectious virus is detected, and they increase in number as the amount of infectious virus increases. They are absent in the late stages of infection. They contain, as does the A body, DNA, RNA, and a nonhistone protein. About 18 hr after infection, the A bodies completely fill the nucleus; at this time, some RNP is extruded from the nucleus to form cytoplasmic RNP bodies (C bodies) in the region of the Golgi apparatus. It is uncertain what these represent and whether virus may be extruded at the same time, by the same mechanism.

#### *Chromosomal Aberrations*

Hamper and Ellison (26) in 1963 reported an increased incidence of chromosome breaks in the MCH line of Chinese hamster cells after infection with herpesvirus. Stich et al. (71), using a virulent and an attenuated strain of herpesvirus in male and female diploid cells of the Chinese hamster and human embryonic lung cells, reported that gaps and breaks in the chromosomes were seen early in the infection, while uncoiling and fragmentation of chromosomes occurred later. The virulent strain led to damage of 90% of the chromosomes at the peak, which was reached by the twenty-eighth hour after infection. They emphasized that the damage was not randomly distributed but that the structural abnormalities were concentrated in "region 3" of the X chromosome and "region 7" of chromosome no. 1, whereas changes in the other chromosomes were not greater than those in the controls.

#### *Reaction of Cells in Tissue Culture*

The different cytological pictures, rounding and giant cell formation, presented by HeLa and rabbit kidney cells infected with the herpesviruses isolated from patients, were described first when these cells were reported to be useful for isolation purposes (61, 69). The possibility that the cytological reaction might be due to different viral strains present in a mixed population was first suggested by Tokumaru (73), working with *Herpesvirus suis* (pseudorabies). Evidence is accumulating that there are two

common forms of cell reaction, and a less common, intermediate form, each of which is induced by a separate strain of *H. hominis*. The cytological pictures, first described by Gray et al. (24), have been repeatedly observed with minor modifications (28, 49, 63). The cytological effects are as follows. The cells become rounded and pile up as loosely attached cells on the surface of the monolayer (P strain of Gray et al., mP strain of Hoggan and Roizman); syncytial giant cells are produced as the result of an infected cell's attracting and fusing with surrounding uninfected cells; two forms occur, a diffuse area of lysis in which hundreds of nuclei are collected about the middle of the area [fR strain of Kohlhage and Siegert (39)], or large distinct giant cells, each containing dozens of nuclei, attached to one another by thin protoplasmic bridges (GC strain of Gray et al., MP strain of Hoggan and Roizman, rR strain of Kohlhage and Siegert); and simple rounding of cells without piling up [NP strain of Gray et al., I (intermediate) strain of Schneeweis]. Small giant cells, with fewer than ten nuclei, are occasionally seen accompanying both P (mP) and NP (I) strains. The type of host cell infected by these virus strains modifies somewhat the actual cytological changes observed. Nevertheless, the cytological change induced appears to be genetically controlled in that the strain of virus breeds true in the same cell line for many generations. However, passage through heterologous hosts or conditions of storage leads to a change in the cytopathic effect, favoring the appearance of the GC form.

When the characteristics of the GC and P strains were measured by other common parameters, they could not be distinguished from one another; their rates of adsorption and of growth in rabbit kidney cells were the same, the pock sizes on the chorioallantoic membrane (CAM) were similar, as was the virulence in mice and rabbits, and, by standard tests, there was complete cross-neutralization between them (65). However, Roizman and Roane demonstrated slight differences between their MP and mP strains in HEp-2 cells. The mP strain is slightly denser than the MP strain, 1.271 to 1.260 g/ml (55); is eluted from a brushite column by 0.55 M sodium phosphate as compared with 0.60 M for MP strain; and, when the kinetics of neutralization with specific mP and MP antisera were measured, a mixture of the two strains

could be separated by finding that there was more rapid neutralization with the homologous than with the heterologous serum (54).

Kohlhage (38) studied in detail the two types of GC response described above. He found that the "rR" strain had the same density as the MP strain, namely, 1.2614 g/ml, while the "fR" strain was lighter, 1.2539 g/ml. He also found that "rR" strain was eluted from an ECTEOLA column at a lower salt molarity than was the "fR" strain. Combining the observations of Roizman and Roane and Kohlhage, it would appear as if the cytological reactions were based on changes in surface structure of the virus strains. The denser strains are associated with the P-type reaction, and the somewhat lighter strains are associated with the usual GC reaction; the lightest strains produce the diffuse lytic reaction. The difference in density has been speculatively ascribed to the lipid content (54).

The mechanism of the large syncytial giant cell formation was studied microcinematographically by Barski and Robineaux (6). The infected cell attracts to itself neighboring uninfected cells, their membranes adhere and fuse, and the uninfected nuclei develop inclusion bodies and collect in the center of the cytoplasmic syncytium. The formation of the small giant cell, seen in the strains that do not form syncytia, was ascribed by Love and Wildy (41) to a partial reconstruction of the mitotic distortion caused by herpesvirus infection. They also suggested that failure to incorporate a damaged chromosome could result in nuclear budding or "amitotic" division. Amitotic division was advanced as an explanation by previous workers on the basis of histological observations (24, 33).

The formation of the syncytial giant cell, characteristically associated with the development of intranuclear virus, must depend on a change in the surface membrane of the infected cell so that it may adhere and fuse with its neighbors. This change does not seem to be governed by the multiplication of the virus in the nucleus but by a separate mechanism, since Nii and Kamahora (50) reported the formation of giant cells without the development of intranuclear inclusions in mouse fibroblasts (L cells). The change followed massive inoculation of the cells with a GC strain virus inactivated by ultraviolet light, but did not follow an inoculation of a nonsyncytial forming

virus similarly treated. They suggested that the GC strain must contain something which affects the cell membrane and which can act when the cells are coated with inactive virus. Further evidence of a specific mechanism for the syncytial formation in HeLa cells was given by Munk and Sauer (46), who found that mitomycin C prevented the development of intranuclear virus but not the development of syncytial giant cells.

## SEROLOGY

### *Serological Epidemiology*

The serological reactivity of *H. hominis* has been the object of study for many years in relation to diagnosis and epidemiological survey. One of the largest surveys, a study of 352 sera taken from subjects of all ages, was reported by Yoshino et al. (89) in 1962. These authors confirmed the trends, noted in previous studies, that babies less than 4 months old had higher antibody levels than children above that age, and that between the ages of 5 and 20 years the frequency of sera which contained antibody increased. During this period titers varied extensively, whereas in patients over 20 years of age the antibody titers tended to be high or absent, the "all or none" phenomenon of the early workers. Although in general there was a close parallelism between neutralizing and CF antibodies, some adult sera had high titers of neutralizing antibody without any detectable CF antibody. On the other hand, some sera of the young children contained no neutralizing antibodies but did contain CF antibodies to the viral (V), but not the soluble (S), antigen, which were heat-labile (60 C for 5 to 30 min). Such heat-labile antibodies were not found among older children or adults. On the basis of these findings, the authors suggested that the earliest infections by the herpesvirus lead to the production of abundant CF antibodies, most of which are comparatively heat-labile. It is only after repeated exposures, as noted by Buddingh et al. (12), that a persistent infection develops which results in the usual finding in adults in whom the CF and neutralizing antibodies quantitatively parallel each other. In the absence of a persistent infection, the antibodies drop or disappear between reinfections. Because the CF antibodies tend to drop sooner than the neutralizing antibodies, some adults could have a positive neutralizing and a negative CF anti-

body titer, depending on the time the blood happened to be drawn. Yoshino and Taniguchi (88) demonstrated that rabbits produce neutralizing antibodies as early as the third day after immunization or infection, which differ from those produced later by their requirement of at least 5 hemolytic units of complement for their detection. Although the later antibodies could neutralize the virus in the absence of complement, the addition of complement enhanced the speed of neutralization. This seemed to hold true also for human sera, in which the addition of complement enhanced the neutralizing power by about twofold. It is worth noting that the techniques used by Yoshino and his co-workers for these studies have many advantages in economy and speed of handling. The use of the 1-day-old egg enables the presence or absence of neutralizing antibodies to be detected in 5 days, because in the presence of infectious virus the blastoderm ceases to grow (87). The titration of the neutralizing activity of each serum can be performed on a chick embryo monolayer growing in a single petri dish (77).

### *Strain Differences*

With the use of the standard type of neutralization and CF techniques, it was questionable whether there were serologically distinguishable strain differences between viral isolates. The early opinion was that the herpesvirus was antigenically homogeneous but, gradually, differences in neutralization and complement fixation titers began to be reported (30). The adaptation of the technique of neutralization kinetics (43) to a study of herpesvirus by Schneweis (64) in 1962 and Ashe and Scherp (3) in 1963 has provided a clear picture of antigenic differences. Schneweis was able to group his 30 strains into two antigenic types, and Ashe and Scherp grouped their 15 strains into four antigenic types and a heterogeneous group.

### *Precipitins*

A further approach to this problem may be the application of the gel diffusion technique for the detection of precipitins. The production of precipitins by *H. hominis* was reported by Tokumaru and Scott (75). An antigen derived from the sonic treatment of rabbit kidney cell tissue culture, infected with a recently isolated strain of herpesvirus, was used to challenge 222

random human sera; precipitin bands, from one to five in number, were detected in 70 sera. These were from patients shown to be convalescent from infection with herpesvirus by standard serological methods. In a selected group of paired sera, precipitin bands were found to develop in some, but not all, convalescent sera

velopement of a tuberculin-like skin test in recovered patients who were inoculated intradermally with heat-killed virus grown in embryonated hens' eggs. This has been confirmed by many workers with antigens from various sources. In 1953 Brown (11) reported a similar observation in guinea pigs. Further analysis of this

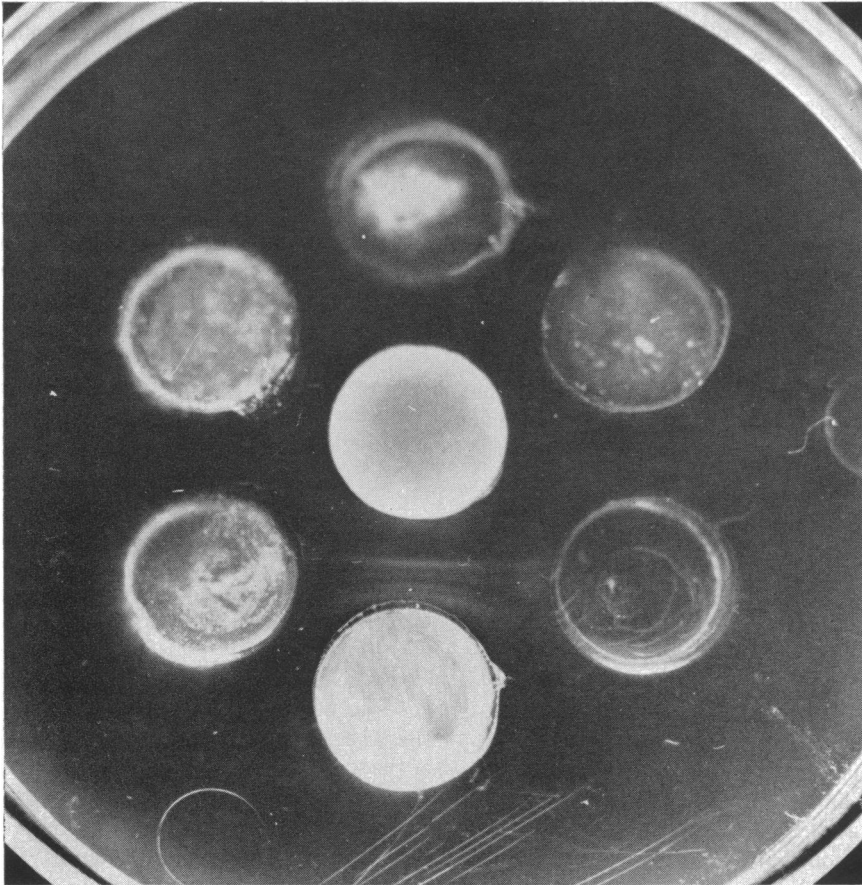


FIG. 1. *Precipitin bands appearing in the convalescent serum collected after a primary herpetic infection. The wells were numbered clockwise, with the topmost well as number 1 and the center well as number 7. First pair: 1 = acute, 2 = convalescent serum. Second pair: 3 = acute, 4 = convalescent serum. Third pair: 5 = acute, 6 = convalescent serum; 7 = antigen well. Note: precipitin bands appeared only in the convalescent serum of the second patient.*

(Fig. 1). Preliminary analysis of the antigen by density-gradient centrifugation in cesium chloride revealed a distinct viral band and at least four distinguishable bands in the soluble fraction (Fig. 2).

#### *Hypersensitivity*

Hypersensitivity to herpesvirus has been recognized since Nagler (47) reported in 1944 the de-

phenomenon was reported by Tokumaru (74) in 1963. In addition to confirming Brown's work, he was able to differentiate by diethylaminoethyl (DEAE)-Sephadex column chromatography three fractions which varied in sensitizing potential. One fraction, eluted in 0.11 M NaCl, had a high sensitizing ability but a low CF activity; another, eluted in 0.27 M NaCl, had a high CF activity and high sensitizing ability; the viral antigen,

eluted in 0.35 M NaCl, had little sensitizing ability but a high CF activity.

#### LATENT INFECTION

The fact of latent infection by *H. hominis* is well known, but the mechanism is still far from clear. The absence of a suitable laboratory model has hindered the acquisition of knowledge in this area, and only a few attempts have been made to study the problem.

operated upon for the relief of the pain of trigeminal neuralgia. Within a few days after section of a branch of the fifth nerve, an extensive eruption of herpetic vesicles frequently appears, which covers the area of the freshly denervated skin. Such eruptions only follow immediately after nerve section since, after a second operation, the vesicles appear in the freshly denervated area but spare the anesthetic area of skin that had followed the previous section. It is a matter

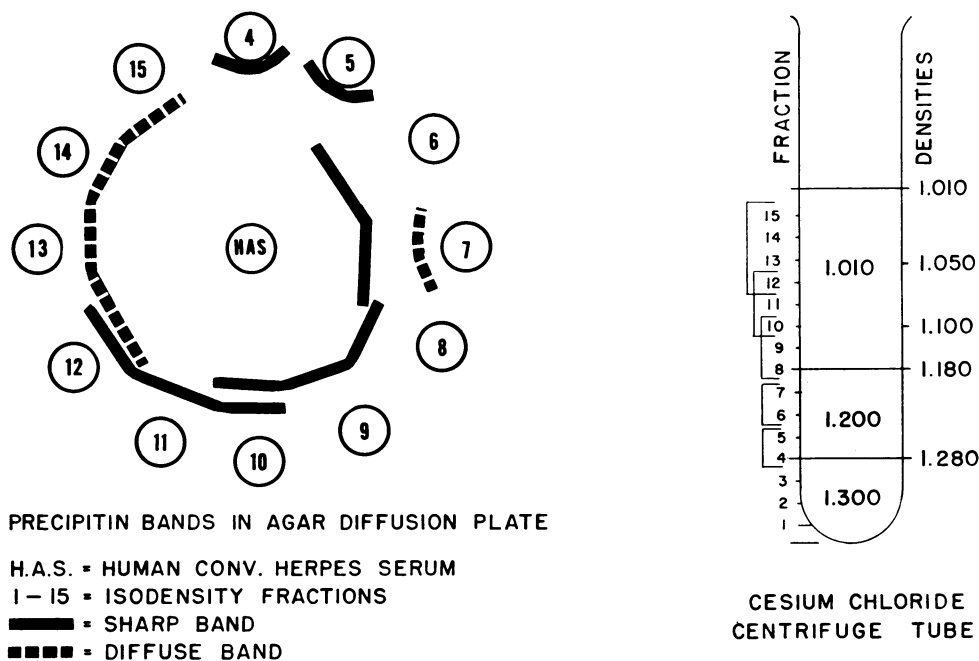


FIG. 2. Precipitin bands in agar diffusion plate. Diagram of precipitin bands after isodensity-gradient centrifugation of *Herpesvirus hominis* antigens.

#### Reactivation

The existence of a latent infection of the brain in rabbits has been recognized since 1938 (52). Rare reports have suggested that reactivation of such a latent encephalitis can be produced in rabbits by anaphylactic shock (23) or by intramuscular injections of adrenalin (62). In relation to the latter, it was speculated that a change in blood supply, producing tissue anoxia, disturbed the balanced host-parasite relationship. Reactivation of herpetic keratoconjunctivitis in rabbits has been reported in 7 of 19 attempts after the induction of Arthus phenomenon in the infected healed eye (1). Reactivation, as a secondary effect of blood supply on local tissue metabolism, is also suggested by the studies of patients

of speculation as to whether the virus lies occult in the nerve ganglion or in the skin. Although no virus has been found in the ganglia, on the few occasions that they have been tested, this clearly does not mean that it may not be there in an occult form. However, it is difficult to conceive how the virus would get to the denervated area of skin to produce the virus-containing vesicles. It seems more probable that the virus lies occult in the skin and is reactivated as a result of the changes in tissue metabolism caused by the denervation (18). The common recurrent lesion is triggered by some disturbance of tissue blood supply, such as fever, ultraviolet light irradiation, or emotion. Following the viremia of the primary infection, the widely distributed virus becomes

occult in the presence of circulating antibodies. The site of reactivation, resultant on local disturbances of tissue metabolism, may be a matter of chance. The frequency of lesions on the face, as opposed to elsewhere on the body, may be accounted for by the greater exposure of the face to exogenous changes involving blood supply to the skin. Special circumstances may determine the site of recurrence, e.g., the truck driver with a recurrent lesion on the back of his thigh, or the postoperative neurosurgical patient with an airway, from whose tracheal secretions reactivated virus may be the source of herpetic whitlow on the fingers of the attendant susceptible nurses (70).

#### *Persistent Infection in Tissue Culture*

How much the observations on persistent infection can be applied to man is uncertain, but there are suggestive analogies to support the speculations in the above section. The disappearance of the infectious virus from the patient roughly coincides with the development of circulating antibodies. The virus either is destroyed or becomes occult. In tissue culture, the standard method of producing a persistent infection is by the addition of antibody to the medium (83). The effect of change in tissue metabolism on the establishment and reactivation of a latent infection was presented by Pelmont and Morgan (51). These authors showed that, when HeLa cells were placed in a nutritionally deficient medium, infection with herpesvirus did not result in the expected cytopathic effect, nor could virus be isolated by passage to fresh cells. However, if the missing nutrients were restored to the infected culture, after several passages in the deficient medium, the virus became detectable and caused the characteristic cytopathic effect. Further evidence along this line could be inferred from the experience of Coleman and Jawetz (16), who found that a modified latent infection could be produced in tissue-culture cells derived from adenocarcinoma of the lung (Maben cells) with one strain (Z strain) of herpesvirus at 31 C. At this temperature, the culture could be kept growing for 9 months, although virus could consistently be isolated on subculture. However, raising the temperature of incubation to 34 C led to rapid destruction of the culture.

#### *Interferon*

Another mechanism contributing to the development of resistance of cells to destruction by herpesvirus is the production of interferon. In 1961 Barski and Cornefest (4) reported that, when a low cancer line of mouse cells (N<sub>2</sub>) was infected with polyoma virus, a latent infection was regularly produced and that these cells resisted further infection both by polyoma and herpesviruses. This they attributed to the production of a virus-inhibiting substance similar to interferon. Glasgow and Habel (22) described a continuous line of mouse embryo cells, persistently infected with polyoma virus (carrier culture 23-P), which partially resisted challenge by herpesvirus. After infection with herpesvirus at low multiplicity, an incomplete cytopathic effect resulted and cells grew out, leading to a double carrier culture that elaborated both polyoma and herpesvirus and was resistant to reinfection by either virus. The equilibrium between the growth of the viruses and the resistance was unstable so that, when the culture was "cured" of its polyoma infection, it was immediately destroyed by the herpesvirus. It was suggested that polyoma and herpesviruses were weak producers of endogenous interferons so that resistance was only achieved by the additive effect of both viruses. A herpesvirus carrier culture could be produced in polyoma-free susceptible cells by adding sufficient exogenous interferon. The production of interferon by the herpesvirus in vivo was demonstrated by inoculating adult guinea pigs with herpesvirus intraperitoneally. These were completely resistant to superinfection by polyoma virus inoculated by the same route within 24 hr and partially, but decreasingly, resistant up to 7 days (5). No therapeutic effect of interferon has been observed on the course of herpetic keratoconjunctivitis in man (15). However, in rabbits, if ultraviolet-irradiated influenza (Lee) virus was applied to one eye within 24 hr after infecting both corneas with herpesvirus, and reapplied four times daily for 4 days, the lesion on the treated eye was much less marked than in the untreated eye, presumably as the result of the interferon produced by the cells infected with the attenuated influenza virus. No benefit was derived from application of the Lee virus after an ulcer had developed (76).

RECENT OBSERVATIONS OF CLINICAL INTEREST  
*Unusual Manifestations of Primary Infection in  
Newborn and Older Infants*

Primary herpetic infection in the newborn is characteristically a serious disease, almost always leading to wide dissemination and death; a similar infection in older infants does not usually lead to clinical evidence of systemic infection. Contrasts to this usual clinical picture have been reported recently.

*Newborn.* Wilson and Martini (86) described a newborn infant with a proven primary herpetic infection whose lesions appeared to be confined to the skin and mucous membranes of the nose and who recovered. The herpesvirus was cultured from the nasopharynx. Scott and Tokumaru observed a similar mild primary infection in an infant born of a mother who was diagnosed clinically as having vulval herpes shortly after delivery of the infant. The infant ran a low-grade fever between the 5th and 14th days, and developed three or four blisters on the right shoulder and about the umbilicus on the 8th day after birth. A virus of low virulence to rabbit kidney cell tissue culture was isolated from the vesicle on the shoulder on the 12th day. The infant made a complete recovery. Both mother and infant showed a significant rise in antibodies, indicating a primary infection in both.

*Older infants.* Becker et al. (7) from South Africa reported a high fatality rate from primary herpetic infection in infants who were suffering from typical Kwashiorkor in their second year of life.

*Mechanisms Whereby the Virus Reaches the  
Nervous System*

Herpetic encephalitis characteristically occurs in one of two forms. In one, the brain seems to be affected as part of a generalized spread of infection, and the virus can be isolated from other organs as well as the brain. In the other, the brain is specifically infected, the other organs being free from the virus. A possible explanation of these different findings may be derived from the recent studies of Johnson (32). He reinvestigated the mechanism of infection of the nervous system for which no entirely acceptable explanation had heretofore been advanced. By the technique of fluorescent-antibody staining, he showed that virus inoculated extraneurally in

suckling mice was concentrated about the small cerebral vessels, indicating that it reached the brain via the blood stream. After intranasal inoculation in the same host, virus could reach the brain by several routes: by direct invasion of the subarachnoid space with dispersion of the virus in the cerebrospinal fluid, the same course as taken after intracerebral inoculation; by invasion of the Schwann cells of the olfactory and trigeminal nerves and centripetal growth from cell to cell; by the blood stream. The virus clearly does not grow along the nerve axons, as has been the most popular but unproven theory in the past. It would seem a reasonable speculation that the isolated central nervous system infection could be via direct invasion, leading to the meningeal form of infection, or by the blood stream, leading to widespread infection of the brain parenchyma, or by both.

*Antiviral Chemotherapy in Herpetic Infection*

The search for antiviral drugs continues in many fields. In 1962 Kaufman (34) reported that 5-iodo-2'-deoxyuridine (IDU) could cure experimental herpetic keratoconjunctivitis in rabbits, and could be successfully used in the treatment of herpetic ocular infections of patients (37). These reports led to widespread, uncontrolled use of the drug, and to the suggestion that a cure rate of 80 to 90% could be expected in the patient with an acute ulcer, but that patients with pathological changes in the deep structures of the eye usually did not respond. Controlled studies in this disease have been complicated by the difficulties encountered in making an accurate diagnosis and the serious course of the disease in some patients, which may limit experimental observation. Two such studies (31, 42) reported no advantage, and one (14) reported a statistically significant advantage from IDU treatment. The author of the last paper used the drug in both acute and chronic herpetic infection, noting improvement only in the patients with acute infections but none among an equally large series of patients with chronic herpetic changes in the eye. The most recent and accurately controlled study was that of Hart et al. (27), who treated 19 patients with IDU and 13 with neomycin drops, every hour during the day and every 2 hr at night for 7 days. By using a table of random numbers to assign the cases to the IDU or the neomycin series, providing rigid criteria for ad-

mitting the patients to the study, limiting the period of study therapy to 7 days, and having only three clinical ophthalmologists caring for the patients in one hospital, they were able to avoid some of the difficulties recognized in the other studies. Under these circumstances, there was a healing response of the ulcer in 74% of IDU-treated eyes and 15% of the neomycin-treated eyes. Failures in treatment have been ascribed to the presence of chronic damage, but also may be due to the emergence of resistant mutants. Such mutants have been reported as occurring in human infection (40). At least one of these has been reported to be susceptible to another antiviral agent, cytosine arabinoside (35), which can produce healing of herpetic keratoconjunctivitis in rabbits even when applied after ulceration has begun (78). Steroids with IDU have been reported to be beneficial (36). The clinical effect of IDU therapy is probably not due to failure of virus multiplication, since, in treated rabbits, despite absence of ulcer, virus can be recovered although in a reduced titer (30a). There do not appear to have been any controlled studies of the use of IDU in herpetic infections of the skin, although it has been reported that herpetic vesicles heal more promptly with IDU (13, 25).

The action of this drug is to inhibit viral reproduction in tissue culture, but the exact mechanism of action is not yet clear. One of its actions is to inhibit thymidine synthetase, but the availability of thymidine from other sources rules this out as the main site of action for viral inhibition by IDU. It is probably incorporated into DNA, leading to a fraudulent DNA that cannot replicate. This is suggested by the observations of Dubbs and Kit (17), who reported that mutants of herpesvirus lacking in thymidine kinase, and unable to induce kinase formation in cells also lacking this enzyme, were completely resistant to IDU, whereas the parent herpesvirus, which induced the appearance of thymidine kinase in this cell line, was inhibited in the presence of IDU. A recent study of Smith and Dukes (68) indicates that the inhibitory action may be on the assembly of the virus. These authors, using the fluorescent-antibody staining technique and electron microscopic examination of uranyl acetate-stained particles, demonstrated that both viral DNA and protein were produced in the IDU-treated cell but that the particles appeared

"ragged, naked and structurally imperfect." The process of viral assembly was discussed by Smith (66), who has shown the presence of naked strings of DNA early in development of the virus. These appear to be folded and encased in the protein capsid, either by an orderly sequential assembly of capsomere subunits or simultaneously. The latter possibility was suggested by the ragged protein coats that are seen on the earliest recognizable viral particles, which are not infectious, as compared with the physically perfect capsids which can be seen when infectious particles are found late in the cycle. The action of cytosine arabinoside does not appear to be on the incorporation of cytosine in the DNA molecule but on a later step in the formation of DNA, possibly at the stage of polymerization.

This review has arbitrarily selected certain aspects of research on herpesvirus for emphasis. The frequent publications concerning this virus suggest that knowledge in this area is increasing rapidly, from which an advance in the general understanding of virus infection can be anticipated.

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